

## OXYGEN UPTAKE IN INSECTS WITH CYCLIC CO<sub>2</sub> RELEASE

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The periodical release of carbon dioxide (CO<sub>2</sub>), as first found by one of us in bugs (Punt, 1944), is well established now in several insects in rest (Punt, 1950, 1956a) and in diapausing pupae of Lepidoptera (Punt, 1950; Schneiderman and Williams, 1953, 1955; Buck, Keister and Specht, 1953; Buck and Keister, 1955).

As to the oxygen uptake, there is some controversy among the above mentioned authors. Punt described in *Carabus nemoralis* (Müll.), *Locusta migratoria migratorioides* (Rch. and Frm.), *Meloë proscarabaeus* L. and *Rhodnius prolixus* (Stål.) a diaferometrically recorded periodical oxygen uptake, which ran parallel to the periodical CO<sub>2</sub> bursts.

In diapausing pupae, on the other hand, a continuous oxygen uptake was found by Schneiderman and Williams and by Buck and Keister using the Warburg technique.

The aim of this paper is to give more details about our investigations concerning the oxygen uptake in carabids and in the pupae of the cecropia silkworm. Some results of experiments on the influence of oxygen tension on the burst frequency will be mentioned. A possible explanation of the oxygen records will be given and the preliminary results of the estimation of the CO<sub>2</sub>-binding power of insect haemolymph will be discussed.

### MATERIAL AND METHODS

The insects under investigation were *Carabus nemoralis* (Müll.), *Hadrocarabus problematicus* Hrbst., *Periplaneta americana* L., pupae of the cecropia silkworm (*Hyalophora cecropia* (L.)) and pupae of *Sphinx ligustri* L.

The cecropia pupae were received from Dr. Schneiderman to whom we want to express our acknowledgment here.

A single animal was put in a glass tube, through which a very constant current of CO<sub>2</sub>-free outdoor air was sucked. The gas exchange was continuously estimated by means of two diaferometers as described earlier (Punt, 1950, 1956a). In these instruments the gas which is to be analyzed passes through a copper tube, in the center of which an electrically heated platina wire is suspended. The temperature of the wire, and as a consequence the electric resistance, depends on the CO<sub>2</sub> and O<sub>2</sub> percentage, respectively, of the passing gas. The wire is connected in a Wheatstone bridge with a galvanometer. In all experiments two parallel diaferometers were used to record the CO<sub>2</sub> production and the O<sub>2</sub> uptake simultaneously. The gas coming from the animal container was dried and equally divided among both machines; the portion for the O<sub>2</sub> diaferometer was absolutely freed from CO<sub>2</sub> by soda lime. As the O<sub>2</sub> reading is slightly influenced by the removal of the CO<sub>2</sub>, a valid R. Q. cannot be extracted directly from the curves. Both galvanometer

responses were recorded on one paper strip in a rotating-drum camera, the slit of which was perpendicular to the direction of rotation. Care was taken that the diaferometers had the same "latent period" in order to get synchronous points on the ordinate of the graphs. The galvanometers were connected in such a way that  $O_2$  uptake and  $CO_2$  release were recorded in the same direction.

In other experiments, for comparison, the direct Warburg method was used to estimate the  $O_2$  uptake. The animals were placed in standard Warburg flasks (16 ml.) containing filter paper drenched in 10% KOH. The temperature was kept constant at 20° C.

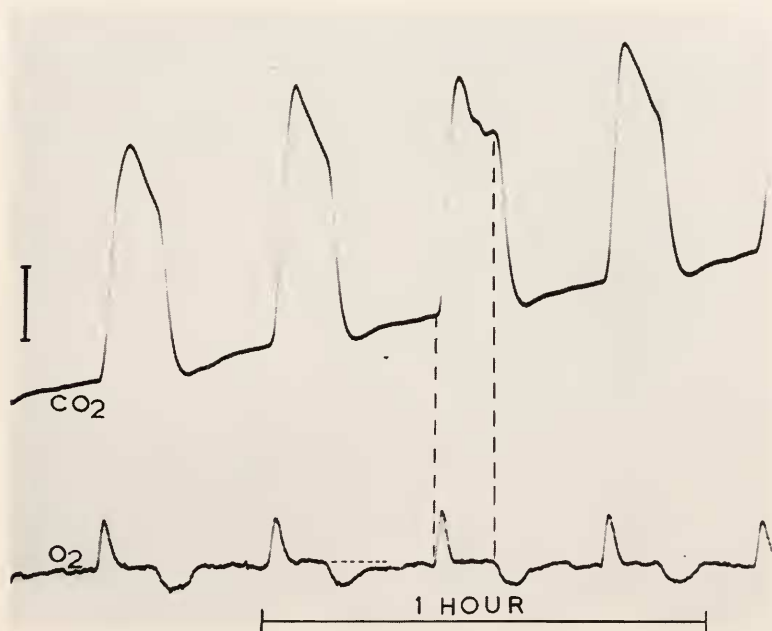


FIGURE 1. Photographically recorded  $CO_2$  production and  $O_2$  uptake of one specimen of *Carabus nemoralis*. The index on the left side indicates approximately  $10 \mu L$  per hour ( $O_2$  and  $CO_2$ ). The inclination of the  $CO_2$  line is caused by galvanometer drift; 20° C.

The  $CO_2$  dissociation curve of haemolymph was estimated by means of the Haldane method for blood gas analysis. In order to work with small quantities, a special apparatus was built with a 10-ml. reaction vessel, the thermo-barometer vessel being of the same size. Gas mixtures were analyzed in the Haldane gas analysis apparatus.

## RESULTS

### 1. Oxygen uptake in carabids

In a previous paper (Punt, 1956a) the discontinuous oxygen uptake in *Carabus nemoralis* (Müll.), *Locusta migratoria migratorioides* (Rch. and Frm.), *Meloe proscarabaeus* L. and *Rhodnius prolixus* (Stål.) was described.

The oxygen uptake was found to be (at any rate partly) periodical; the spikes in the photographic records were exactly synchronous with the  $CO_2$  bursts. But

there could be noticed a marked difference between the forms of the  $O_2$  and  $CO_2$  curves, which difference was most evident in *Carabus* (Figs. 1 and 2).

Both the opening and the closing moments of the spiracles are rather distinct in the  $CO_2$  line: a steep curving up of the galvanometer record indicates the moment of opening and a more or less sharp notch in the line, followed by a decline to zero level (or so close to zero that the difference was within the range of experimental error), marks the moment of closing. Sometimes, in *Carabus* and *Periplaneta*, the line rises somewhat in the interburst period (Fig. 1), probably due to small interburst  $CO_2$  release (*cf.* Schneiderman and Williams, 1955).

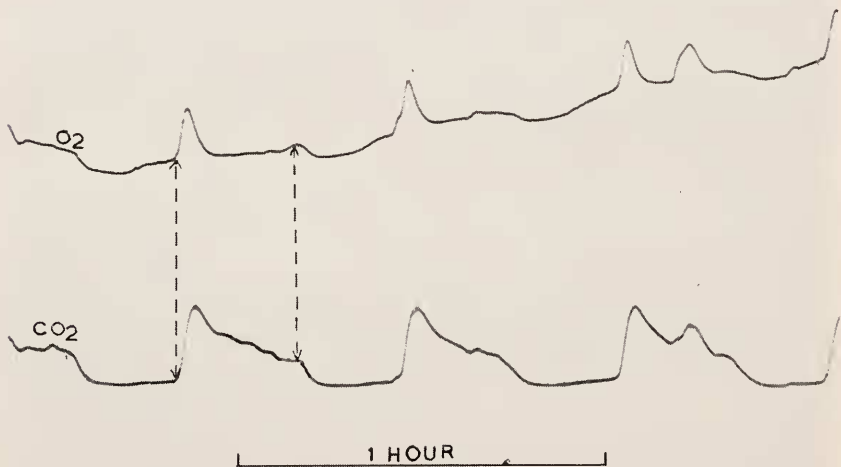


FIGURE 2. As Figure 1, but from a beetle in which the ratio between the open period and the closed period is discrepant from the mean value (1:2) as is occasionally found.

The  $O_2$  line, too, shows these peculiarities, but while the  $CO_2$  spike shows a maximum just after opening of the spiracle and declines rather regularly till the moment of closing, the  $O_2$  line goes down much steeper nearly to the interburst level. After the moment of closing of the spiracles (corresponding to the sharp bend in the  $CO_2$  line) the  $O_2$  line declines still more to a minimum, to curve upwards again after some time to come to a "steady-state" which is maintained till the next burst. This steady rate of oxygen consumption is slightly lower than the minimum  $O_2$  uptake in the burst period. As at that time we only meant to determine the type of respiratory activity and not the total gas exchange, there were unfortunately no zero readings recorded in the above mentioned paper. The respiration was recorded over periods of 6 hours continuously and it is rather inaccurate to interpolate a zero line over so long a period, particularly as there may have been some galvanometer drift due to external circumstances.

Next we performed some experiments of much shorter duration, preceded and followed by zero readings in order to be able to interpolate the zero line in our

records. Though there was considerable individual variation, the  $O_2$  uptake in the interburst periods could be calculated from planimeter measurements to be approximately 60% of the total  $O_2$  consumption. So in *Carabus* the oxygen uptake is both continuous and periodical. In a great number of experiments with *Periplancta americana* we came to the same conclusion. It has already been shown that these  $O_2$  spikes could not be caused by  $CO_2$  interference in the  $O_2$  diaferometer (Punt, 1956a).

As was mentioned, Schneiderman and Williams, and Buck and Keister reported the  $O_2$  uptake in diapausing pupae to be continuous only. In order to determine whether this discrepancy between our results in *Carabus* and the results of Schneiderman and Buck was the consequence of the use of different techniques, the gas ex-

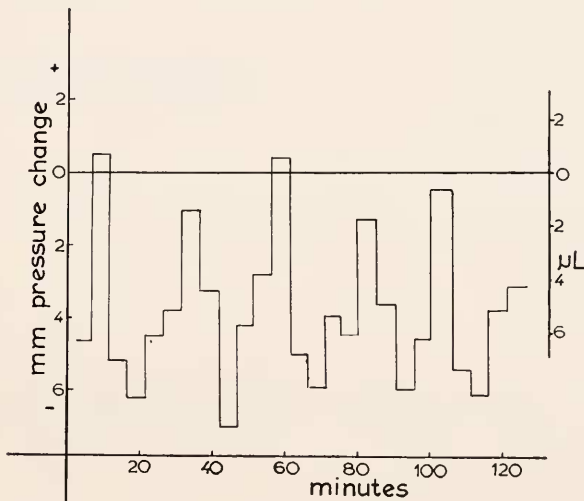


FIGURE 3. Manometrically estimated  $O_2$  uptake in *Hadrocarabus problematicus* (440 mg.). Warburg flasks provided with KOH-drenched paper;  $20^\circ C$ .

change of carabid beetles was estimated in a Warburg apparatus. There were performed a great number of experiments with specimens of *Hadrocarabus problematicus* of a weight of 250–650 mg. The insects were placed in a normal side-arm flask with gas vent. The side-arm contained filter-paper, which protruded into the flask above the beetle. The paper was drenched in 10% KOH. After half an hour of temperature equilibration the stopcocks were closed and manometer readings made at intervals of three or five minutes. The change in pressure in this interval as compared with the foregoing reading was plotted against time (Fig. 3). In this way the oxygen consumption could be calculated from these figures, taking the flask constant into account. As the insect's volume was not exactly known, the index on the right of Figure 3 represents an arbitrary value only. The results show that  $O_2$  consumption in *Hadrocarabus* is at any rate partly periodical, which is in accordance with the diaferometer experiments. Sometimes the curve rises above the zero line, which would indicate that there is in this interval an increase in pressure in comparison with the foregoing reading. This can only mean that at these mo-

ments the  $\text{CO}_2$  bursts occur and the  $\text{CO}_2$  absorption is not yet completed. But as the  $\text{CO}_2$  is readily absorbed, the pressure is considerably decreased in the following five minutes, accentuating the apparent  $\text{O}_2$  uptake in this period. From a large number of experiments with the diaferometer we know that at this temperature ( $20^\circ \text{C}.$ ) in carabids the open spiracle period can be sharply distinguished from the closed period, the interburst period being mostly twice as long as the burst. As this ratio cannot be clearly seen in the manometrical curves it may be concluded that in *Hadrocarabus*  $\text{O}_2$  uptake is partly continuous as well.

When instead of containing KOH-drenched filter paper the side-arms were empty, the line represented in Figure 4 was found. Here, too, the moment of opening of the spiracles could be found in the curve as an increase in pressure,

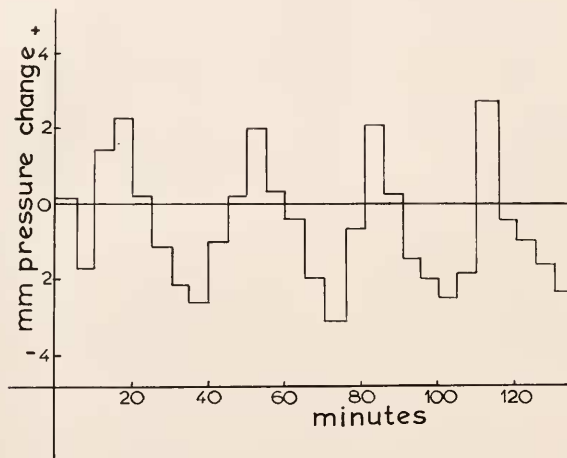


FIGURE 4. *Hadrocarabus problematicus* (500 mg.). Variations in pressure in a Warburg flask, without KOH;  $20^\circ \text{C}.$

reaching a maximum in about ten minutes. This was followed by a slow decrease in pressure, lasting for about fifteen to twenty minutes by which the cycle was completed.

The decrease in pressure in the closed period is interpreted as a continuous  $\text{O}_2$  consumption, but Buck, Keister and Specht (1953) considered the possibility that telescoping of the insect was involved, which in this manometric method could not be distinguished from some other phenomenon causing pressure loss. Our results, however, in the "open circuit" with the diaferometer made this assumption doubtful. Still we thought that in this beetle the space between the abdominal tergites and the elytra, which can be rather well closed and into which eight out of nine pairs of spiracles open, had something to do with the cyclic gas exchange. So we perforated the elytra. This had not the slightest influence on the periodical respiration. The sealing of three pairs of the spiracles with melted bee's wax made the periods more irregular and increased their frequency. It did not matter very much which pairs of spiracles were sealed. Introduction into the spiracles of very tiny glass capillaries, which were fixed with bee's wax, made the  $\text{O}_2$  uptake more con-

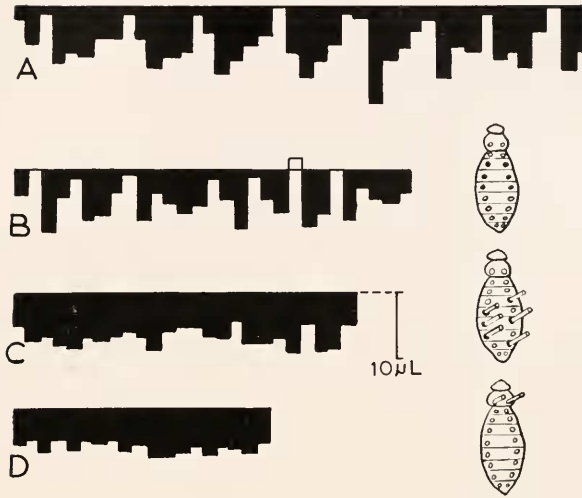


FIGURE 5. Oxygen uptake in *Hadrocarabus*, manometrically estimated at three-minute intervals; 20° C. A: normal insect at rest, the elytra being removed. B: Some of the spiracles sealed with bee's wax. C: Some of the abdominal spiracles kept open with glass tubes. D: The same with the thoracic spiracles.

tinuous. The clearest effect resulted here from placing the capillaries in the large thoracic spiracles (Fig. 5).

## 2. The oxygen uptake of *Hyalophora cecropia* pupae

Two years ago, in the Physiological Laboratory, University of Utrecht, the following experiments were performed with cecropia pupae. The gas exchange was

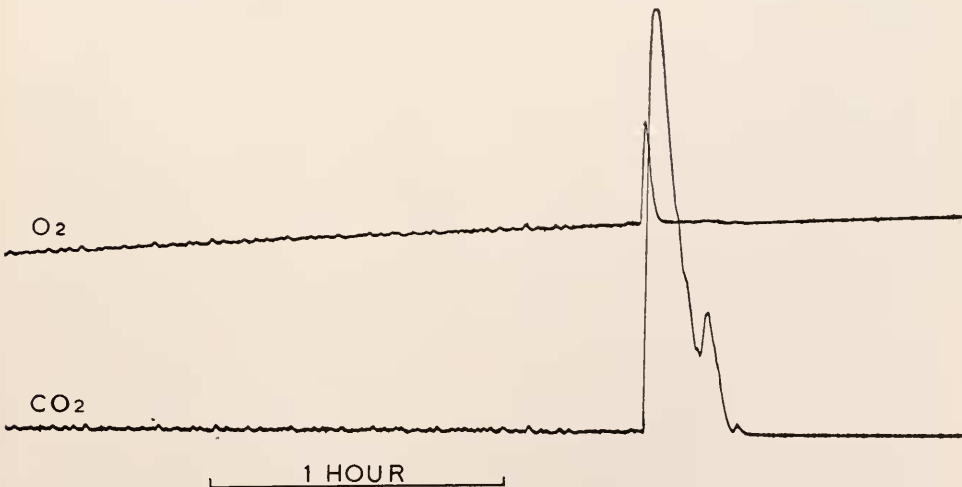


FIGURE 6. Photographically recorded CO<sub>2</sub> release and O<sub>2</sub> uptake in a pupa of the cecropia silkworm; 20° C.

estimated with two diaferometers simultaneously, one for recording the  $\text{CO}_2$  production, the other for the  $\text{O}_2$  consumption. In Figure 6 the photographically recorded galvanometer responses are shown. At  $20^\circ \text{C}$ . these diapausing pupae showed one  $\text{CO}_2$  burst in about four hours. The form of the  $\text{CO}_2$  line is exactly the same as described earlier in pupae of *Sphinx ligustri*. Just after the  $\text{CO}_2$  spike the line falls to approximately zero level and is very smooth, but after about one hour small perturbations return in the line, which go on and increase until the next  $\text{CO}_2$  burst. Exactly synchronous with the  $\text{CO}_2$  bursts small  $\text{O}_2$  spikes were found. This  $\text{O}_2$  line falls after some minutes but remains slightly above the interburst level. In the interburst period the  $\text{O}_2$  line, too, is at first very smooth, but shows after some time the same small perturbations as the  $\text{CO}_2$  line (in fact running exactly parallel to them). It looks as if the spiracles are no longer hermetically closed but are leaking or fluttering from time to time. Unfortunately we did not esti-



FIGURE 7. The same pupa as in Figure 6. A double burst is recorded.

mate the exact value of the  $\text{O}_2$  uptake in these prolonged experiments, as was pointed out in the foregoing section. But as the  $\text{O}_2$  diaferometer was adjusted to be approximately as sensitive as the  $\text{CO}_2$  apparatus in order to get the same response for the same percentage of gas, it is clear from these graphs that  $\text{O}_2$  uptake must take place in the interburst periods as well. The area of the  $\text{O}_2$  spike was much smaller than that of the  $\text{CO}_2$  burst, whereas the interburst perturbations were of the same size in both lines. The impression which we have from some zero readings of the  $\text{O}_2$  line and from calculations of the  $\text{O}_2$  spike area is that there must be a relatively large interburst  $\text{O}_2$  uptake which was considered to have a mean value of 92% of the total  $\text{O}_2$  uptake. This confirms the findings of Schneiderman and Williams and of Buck *et al.* But still a periodic  $\text{O}_2$  uptake is found as well. Perhaps of interest in this connection is what we find on page 147 in the paper of Buck and Keister (1955) where we can read: "Small but statistically significant perturbations were in fact seen in some of our  $\text{O}_2$  uptake records. . ."

Occasionally a double burst was recorded as was already described for pupae of *Sphinx* and *Papilio* (Punt, 1950; Fig. 7).

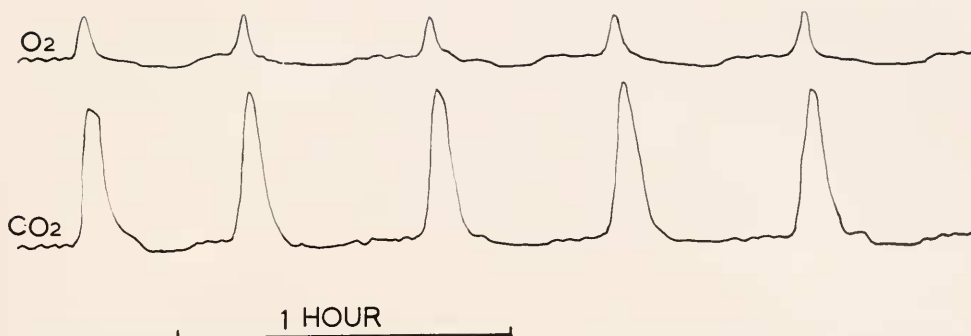


FIGURE 8. Gas exchange of a cecropia pupa, a few weeks before hatching (May, 1954).  
 Drawn from photographic records.

In Figure 8 we find a record of simultaneously estimated  $\text{CO}_2$  release and  $\text{O}_2$  uptake of the same cecropia pupae made a few weeks before hatching. The graph is on the same amplification and on the same time scale as Figure 6. The burst frequency has increased to two per hour. The  $\text{O}_2$  uptake in the bursts is relatively larger.

### 3. Influence of oxygen tension on burst frequency

The influence of  $\text{CO}_2$  tension on the burst frequencies in *Carabus* has been previously described (Punt, 1955, 1956b). Increasing  $\text{pCO}_2$  caused a prolonged open-

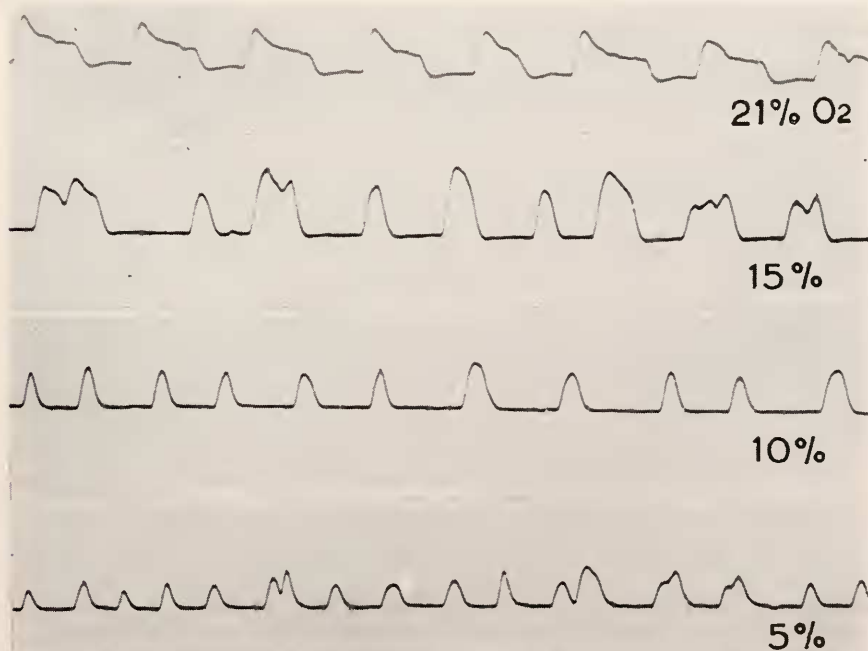


FIGURE 9. *Hadrocarabus problematicus*;  $\text{CO}_2$  burst frequency as influenced by different  $\text{O}_2$  tensions.



ing of the spiracles, until at about  $1\frac{1}{2}\%$  of  $\text{CO}_2$  the closing of the spiracles was inhibited. In the present investigation experiments were performed on the influence of decreasing oxygen tension on burst frequency in *Hadrocarabus problematicus*. In Figure 9 a summary is given of the results. A decrease of  $p\text{O}_2$  caused an increase in  $\text{CO}_2$  burst frequency and consequently a decrease in burst volume. This is in accord with Wigglesworth's observation on spiracular movement in fleas (Wigglesworth, 1953).

#### 4. The $\text{CO}_2$ dissociation curve of haemolymph

For reasons given in the discussion, we thought it worthwhile to know something about the  $\text{CO}_2$  dissociation curve of insect haemolymph. Much work has been done on the composition of the body fluid of insects, but as to the possibility of buffering the  $\text{CO}_2$  our knowledge is scanty (Reali, 1955; van Asperen and van Esch, 1956; Levenbook and Clark, 1950). We therefore tried to estimate the  $\text{CO}_2$

TABLE I  
The  $\text{CO}_2$ -binding property of haemolymph of *Sphinx ligustri* pupae

Quantity of haemolymph (mL)	$p\text{CO}_2$ (mm.Hg)	Total $\text{CO}_2$ (volume %)
0.50	0	$2.2 \pm 0.8$
0.70	0	$1.7 \pm 0.6$
0.69	0	$2.2 \pm 0.6$
0.49	19	$5.1 \pm 0.9$
0.23	31	$10.9 \pm 1.0$
0.50	38	$9.2 \pm 1.1$
0.53	38	$8.9 \pm 0.9$
0.38	73	$18.2 \pm 1.4$
0.24	84	$17.9 \pm 2.2$
0.33	105	$20.0 \pm 1.5$

saturation of haemolymph under different tensions of  $\text{CO}_2$ . The haemolymph of diapausing pupae of *Sphinx ligustri* was gathered by puncturing the pupae with a syringe which was previously moistened with tromboliquine (heparin), in order to prevent clotting. The exactly measured quantity of haemolymph was put in the small bulb-flask of the Haldane blood-gas analyzer and saturated with  $\text{CO}_2$  under a certain tension by rotating the flask mechanically for one hour. After that time the analysis was performed in the normal way, tartaric acid being used to expel the  $\text{CO}_2$  from the haemolymph. The results are to be found in Table I and in Figure 10. The data are corrected for dissolved  $\text{CO}_2$  at the different tensions and at the temperature used ( $20^\circ \text{C.}$ ) so that the total amount of  $\text{CO}_2$  could be plotted against the  $p\text{CO}_2$ . Though only a few experiments were performed (our stock of pupae being exhausted for this season), it proved that the  $\text{CO}_2$ -binding capacity exceeds the line which represents the dissolving of  $\text{CO}_2$  in water at the same temperature (the solid line in Figure 10; data from Umbreit *et al.*, 1949). Our data are too few in number to allow drawing a correct line through the points.

Insect haemolymph probably contains some  $\text{CO}_2$ -binding principle but in our pupae the capacity was rather disappointing as only twice as much  $\text{CO}_2$  could be extracted from the haemolymph as would dissolve in pure water. In certain other

insects (*Gastrophilus* and *Hydrophilus*; cf. Florin, 1937) the quantity of bound  $\text{CO}_2$  was found to be much larger, but these may perhaps represent special cases. More work on this subject is in progress.

## DISCUSSION

We will try to give a possible interpretation of the recorded  $\text{O}_2$  uptake and  $\text{CO}_2$  release in *Hadrocarabus*. This interpretation is, of course, only hypothetical, but

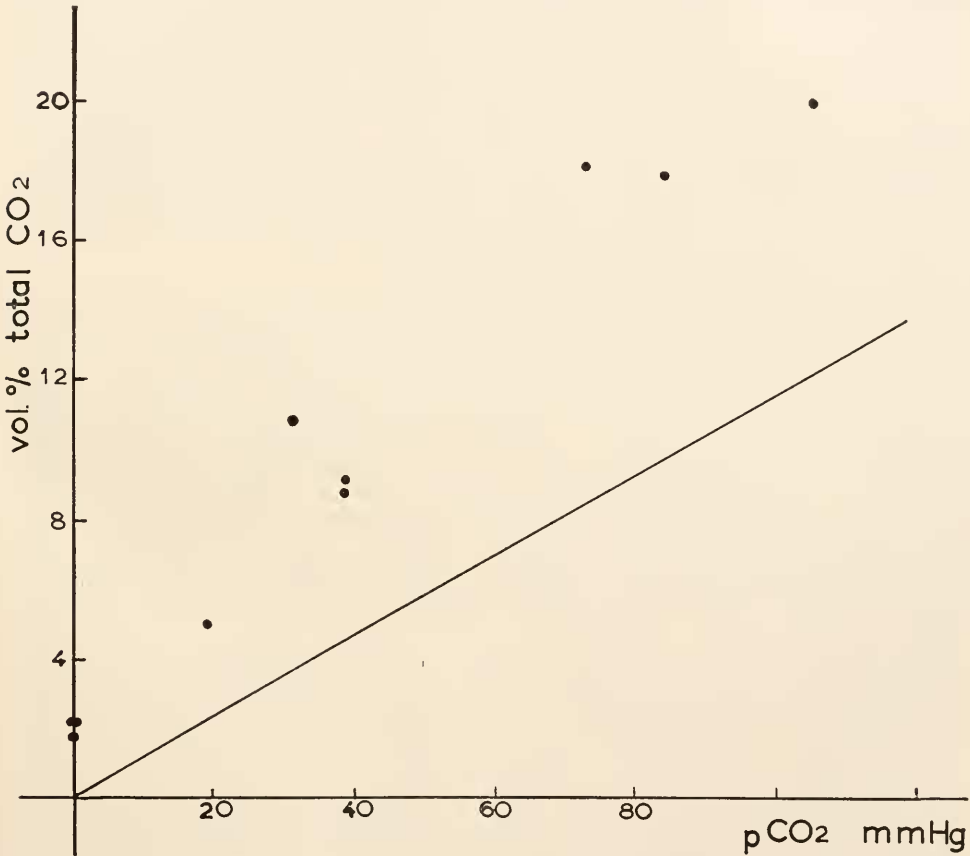


FIGURE 10. The  $\text{CO}_2$  capacity of haemolymph of *Sphinx ligustri* ( $20^\circ \text{C}$ ). Solid line:  $\text{CO}_2$  capacity of pure water.

is in agreement with the results of our and other investigations and partly based upon assumptions from Wigglesworth (1953) and from Buck and Keister (1955, p. 162). Probably in other insects, including diapausing pupae, the same phenomena may occur.

We may assume that oxygen is taken up continuously in some way or another. But the quantity which can penetrate into the insect body in the "closed spiracles"

period is insufficient to cover the metabolic oxygen want. So an oxygen debt is incurred, oxidation being incomplete, and acid metabolites are formed and accumulated in the body fluid. This is in accordance with the theory of Wigglesworth, who attributes the withdrawal of fluid from the tracheal endings to increased osmotic value of the tissue fluids, due to the increasing amount of acid metabolites.

Carbon dioxide is formed by metabolic processes and stored in the body fluid, not only as dissolved gaseous  $\text{CO}_2$  but probably bound in some buffer system or  $\text{CO}_2$ -binding principle. But as soon as the  $p\text{O}_2$  in the tracheal system is diminished and an oxygen debt develops in the tissues, the acid metabolites drive the  $\text{CO}_2$  from the bound phase into the gas phase and as a consequence the  $p\text{CO}_2$  in the tracheae will increase. As soon as the  $p\text{CO}_2$  has reached a certain threshold the spiracles open and the  $\text{CO}_2$  burst takes place. In the open period the  $\text{CO}_2$  diffuses out, not only from the tracheae but from the tissue fluid as well, until the moment when the  $p\text{CO}_2$  reaches a level at which the spiracles may close. In the meantime  $\text{O}_2$  has entered the tracheae, which is seen as the initial spike in the  $\text{O}_2$  uptake line. But after this initial spike the  $\text{O}_2$  line falls down to a level slightly above the steady state of the interburst period. Probably this part of the  $\text{O}_2$  line represents the real oxygen consumption of the animal per unit of time.

When the spiracles are closed at last, the amount of  $\text{O}_2$  in the tracheal system is sufficient to cover the metabolic want for some time (the  $\text{O}_2$  line going down to nearly zero), but as soon as the  $p\text{O}_2$  inside the trachea is low enough, a leakage of  $\text{O}_2$  starts (continuous  $\text{O}_2$  uptake). This leakage is not sufficient, however, to cover the real oxygen want and acid metabolites are formed again, by which the described respiratory cycle is completed. This hypothesis is in accordance with the assumption that  $\text{CO}_2$  controls the sudden opening of the spiracles (Buck and Keister, 1955; p. 161). The action of decreased  $p\text{O}_2$  on the triggering of the spiracular opening may be seen as an indirect one: decreased  $p\text{O}_2$  increases the degree of hypoxia and hence the amount of gaseous  $\text{CO}_2$  liberated into the tracheae. Similarly Wigglesworth's assumption that in pure oxygen, opening is induced by a large amount of  $\text{CO}_2$ , and in 5%  $\text{O}_2$  by a very small amount of  $\text{CO}_2$ , could be interpreted to mean that in pure oxygen the  $\text{CO}_2$  remains bound in the body fluid and that in both cases (100% of  $\text{O}_2$ , and 5% of  $\text{O}_2$ ) the tension of free  $\text{CO}_2$ , triggering the spiracles, may be the same. Probably this threshold  $\text{CO}_2$  tension is not very high, for there is evidence that in about 1½% of  $\text{CO}_2$  the spiracles do not close at all (Punt, 1956b).

So it looks as if in all cases it is the  $p\text{CO}_2$  which controls spiracular movement. When oxygen tension is lowered, oxygen debt develops more readily and the  $p\text{CO}_2$  will reach the critical level sooner. As a consequence the burst frequency is increased. The burst volume is decreased, as no large quantities of  $\text{CO}_2$  can be accumulated in the short interburst period. It would be worthwhile to determine the pH of the haemolymph under these circumstances. When oxygen tension is raised, acid metabolites are not formed so soon and more metabolic  $\text{CO}_2$  may be bound in the body fluid, the intertracheal  $p\text{CO}_2$  only reaching the critical value after a longer period; burst frequency is decreased, burst volume increased (Punt, 1956b). As to the experiments in pure oxygen, when the spiracles are opened the tracheae are filled with pure oxygen and as a consequence the oxygen leak in interburst period is much less. Schneiderman and Williams (1955; p. 134) stated that the "interburst  $\text{CO}_2$  output," too, may become undetectable in pure oxygen.

## SUMMARY

1. The simultaneous determination of  $\text{CO}_2$  production and  $\text{O}_2$  uptake in *Hadrocarabus problematicus* Hrbst. is described. Both  $\text{CO}_2$  release and  $\text{O}_2$  uptake are cyclic, but there is some interburst  $\text{O}_2$  uptake.

2. The diaferometrically recorded  $\text{O}_2$  uptake is discussed, and compared with the results as obtained with the Warburg technique.

3. In diapausing pupae of the cecropia silkworm (*Hyalophora cecropia*) the continuous  $\text{O}_2$  uptake forms a larger percentage of the total amount of  $\text{O}_2$  consumption than in *Hadrocarabus*. Still there was found an initial maximum of  $\text{O}_2$  uptake of short duration at every  $\text{CO}_2$  burst.

4. Some experiments on the  $\text{CO}_2$  dissociation curve of haemolymph of pupae of *Sphinx ligustri* are mentioned. There is evidence that in haemolymph a  $\text{CO}_2$ -binding principle is present.

5. The interaction of  $\text{O}_2$ , haemolymph and  $\text{CO}_2$ , resulting in a certain intertracheal  $\text{pCO}_2$  controlling spiracle movement, is discussed. Probably the  $\text{pO}_2$  is only indirectly involved in the triggering of spiracle opening.

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